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13. ABSTRACT (Maximum 200 Words) Our previous work demonstrated that prostate cancers differ from benign prostatic epithelium in their expression of oncogenic members of the pp32 gene family. Whereas benign prostatic epithelium solely expresses pp32, a tumor suppressor, prostate cancers express pp32r1 and pp32r2, which are oncogenic. The purpose of the study is to confirm and extend these preliminary results, to develop practical means to assay pp32 gene family members in clinical samples, and to determine the clinical significance of their presence. The approved proposal encompassed four broad tasks: [1] characterization of the pp32 expression phenotype of a larger sample of 40 prostatic adenocarcinomas; [2] development of a practical molecular pathology assay for altered pp32 transcripts; [3] adaptation of the assay to paraffin-embedded tissue; and [4] preliminary determination of the clinical utility of pp32r1 and pp32r2 expression in prostatic adenocarcinoma. In the course of pursuing this work, we recognized that improved assay methods would yield better results for Task 1. We have completed development of a robust and practical molecular assay (Task 2) and have nearly completed Task 3, adaptation to paraffin. In the remaining funding period, these tools shall be applied to completion of Task 1 and Task 4.			
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INTRODUCTION

Our previous work demonstrated that prostate cancers differ from benign prostatic epithelium in their expression of oncogenic members of the pp32 gene family. Whereas benign prostatic epithelium solely expresses pp32, a tumor suppressor, prostate cancers express pp32r1 and pp32r2, which are oncogenic. The approved proposal encompassed four technical objectives: [1] characterization of the pp32 expression phenotype of a larger sample of 40 prostatic adenocarcinomas; [2] development of a practical molecular pathology assay for altered pp32 transcripts; [3] adaptation of the assay to paraffin-embedded tissue; and [4] preliminary determination of the clinical utility of pp32r1 and pp32r2 expression in prostatic adenocarcinoma.

BODY

- Task 1. This approved task involves characterization of abnormal pp32 transcripts in frozen samples of human prostatic adenocarcinoma compared to paired normal prostate controls. 40 pairs of prostatic adenocarcinoma and normal prostate are to be analyzed to determine the range and frequency of occurrence of pp32 gene family-related sequences in prostatic adenocarcinoma.
- Progress: Work on this task has been deferred pending development of the assay outlined in Task 2. Now that Task 2 is complete, and Task 3 is nearly complete, work is scheduled to commence on Task 1 within the next several weeks.
- Task 2. This approved task aims at development of a practical molecular pathology assay to distinguish individual members of the pp32 gene family. Briefly, this task involves selection and optimization of PCR primer sets for efficient amplification of altered regions of pp32. The original objective was to select and optimize restriction enzyme cleavages to distinguish among normal pp32 and the various altered forms of pp32. This involved standardization of the assay using defined mixtures of plasmid DNA to determine sensitivity and specificity under optimized PCR conditions and comparison of assay performance on known samples of RNA from frozen tissue.
- Progress: This task is complete. Unanticipated difficulties were encountered in executing the plan for this task as initially proposed. The sequences of the known members of the pp32 gene family, pp32, pp32r1, and pp32r2, contain a number of features that complicate their assay: the sequences are approximately 90% identical; the sequence differences are largely not clustered; the sequences possess high GC content; and finally, amplification of single PCR.

Successful completion of this task was accomplished by the following strategy: [a] specific primers were developed for pp32, pp32r1, and pp32r2 for use in RT-PCR; [b] following amplification, the resultant product is slot blotted onto a nylon membrane; [c] the membrane is then hybridized with a labeled oligonucleotide specific for pp32, pp32r1, or pp32r2; and [c] X-ray film captures the image of the hybridized, chemiluminescent slot blot. Figure 1, below, shows the specificity of this assay.

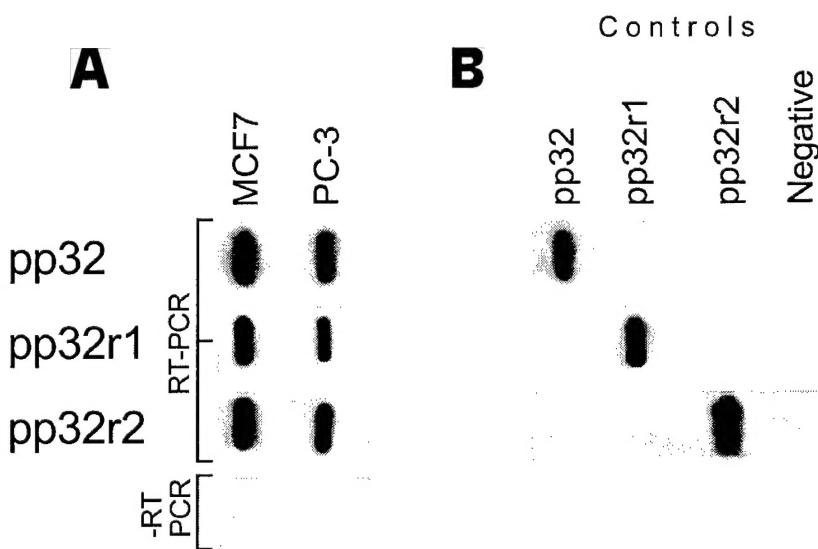


Figure 1. Specific Detection of mRNA encoding pp32, pp32r1, and pp32r2. Panel A shows the results obtained by RT-PCR and slot blot hybridization of either MCF7 mRNA or PC-2 human prostatic adenocarcinoma mRNA, and Panel B illustrates the same experiment performed with equal amounts of controls consisting of either pp32 mRNA, pp32r1 mRNA, pp32r2 mRNA, or no mRNA ("Negative"); each control mRNA species was prepared by *in vitro* translation of the corresponding plasmid. The specificity of the reaction carried out in each horizontal lane is indicated in the left-hand column.

It should be noted that this assay was extremely difficult to develop. The following technical difficulties had to be overcome: [a] the high degree of similarity of pp32 gene family mRNA species; [b] high GC content of all species; and [c] the high degree of conservation of 5' and 3' untranslated regions; and [d] the general lack of clustering of sequence differences among pp32 gene family species.

In work that is beyond the scope of the present project and is mentioned herein for informational purposes only, the assay shown above is being further developed into a quantitative assay that will be fully capable of reproducible determination of the level of pp32 gene family mRNA expression in terms of the number of copies of each message present per

cell.

Task 3. This approved task aims to adapt the molecular pathology assay for use archival tissue. Briefly, the task involves preparation of RNA from set of paraffin-embedded human prostatic adenocarcinomas, paired adjacent normal prostates, and control tissues. This is followed by amplification of pp32 mRNA by RT-PCR and analysis by the assay developed in Task 2, and validation of the assay by subcloning and sequencing of selected regions or entire inserts as indicated, using methods described for Task 1.

Progress: The assay outlined in Task 2 was specifically designed to be compatible with RNA fragments harvested from paraffin. The amplicons for pp32, pp32r1, and pp32r2 are each in the 300 to 350 bp range, which is generally compatible with the size of RNA fragments extractable from paraffin-embedded tissues. Work is currently in progress to determine the performance characteristics of the assay on paraffin-embedded tissue. Completion of this task is estimated to occur within two to four weeks.

Task 4. This approved task seeks a preliminary determination of the clinical significance of pp32 molecular changes. This task requires assembly of paraffin blocks from previously studied population of prostatectomy specimens (1), preparation of RNA from paraffin sections, analysis of pp32 RNA by molecular assay developed under Tasks 2 and 3. The results will be selectively validated by selective subcloning and sequencing, as described in Task 1.

Progress: Work on this task is scheduled to begin as soon as Task 3 is complete, i.e. within several weeks.

KEY RESEARCH ACCOMPLISHMENTS

Developed reliable and robust molecular assay to distinguish pp32, pp32r1, and pp32r2 mRNA's from one another in tissue samples. These molecules are ~90% identical to one another, possess high GC content, and have conserved 5' and 3' untranslated regions, making this a highly significant technical accomplishment (Task 2)

Have almost finished adapting the assay to paraffin-embedded material (Task 3)

REPORTABLE OUTCOMES

None at present. Anticipate submission of manuscript in preparation in Q2, 2001.

CONCLUSIONS

The development of a reliable molecular assay to distinguish pp32 gene family mRNA's from one another in clinical material is anticipated to have great value not only to this project and the remaining approved tasks, but to the prostate cancer field in general. Recent publications by other laboratories entering the pp32 field link pp32 to regulation of stability of immediate-early mRNA species containing AU-rich elements (2) and to a complex that inhibits histone acetyl transferase, affecting gene expression (3). The assay together with identification of other possible expressed gene family members in the near future is expected to accelerate translation of work in this area into diagnostic assays and potential therapeutic targets that should be of great benefit to the prostate cancer community.

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APPENDICES

None